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"OPTIMIZATION OF GROWTH CONDITIONS FOR SPAWN PRODUCTION OF *PLEUROTUS FLORIDA*"

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ABSTRACT

Gilled mushrooms are widely accepted as the edible mushroom. Oyster Mushroom or Pleurotus Florida are commonly cultivated around the world. Fungi(Pleurotus) showed dits role as my co-remediation for pollutants degradation similar to polycyclic aromatic hydrocarbons and petroleum. Spawns are the seed extracted for growing mushrooms; they are vegetative mycelium of oyster mushroom cultured in Potato Dextrose Agar medium with grain as secondary food or Nutrition such as wheat, Black Gram, Maize, Pea grains etc. However, spawn are grains covered with mushroom mycelium. An effective result was obtained at Potato Dextrose Agar media with fresh mycelium and High yield grains of Wheat. Bottle of Spawn filled with Boiled wheat's up to 70% along with 5% of the mycelium of test oyster mushroom. Conditions allotted included sterilization with UV for one hour and autoclaving of Bottles for my celium inoculation followed by maintaining the temperature of 20 ^{0}C for 20 days to obtain Mother culture. Subsequent transfer of 30% of mother spawns to boiled wheat grains for 15 days incubation results in sister culture and 11 days for planting culture.

KEYWORDS: Pleurotus Florida, Mycoremediation, Spawn & Planting Culture

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Original Article

INTRODUCTION

Oyster Mushrooms or the gilled mushrooms are composed and compromised for one of the widely edible mushrooms Such as *P. Ostreatus*. *Pleurotus* species are also known as oyster or a balone, or tree mushrooms is cultivated as edible mushrooms across the world. *Pleurotus* fungi show application in myco-remediation of water and air pollutants such that it comprises petroleum and polycyclic aromatic hydrocarbons. *Pleurotus* is also referred to as "side ear". Caps are laterally attached to the stem. *Pleurotus* is commonly eccentric and gills seems to decurrent with it. *Pleurotoid* is a term used for those mushrooms which have general shapes. Spores of these mushrooms are smooth and elongated. Hyphae of it meet and are joined by clamp connections. *Pleurotus* are not considered to as bracket fungus but plenty of the species were found to be monomitic along with a soft consistency. Somehow, *Pleurotus Dryinus* are dimictic considered as it has additional skeletal hyphae proving a tougher consistency referred to as bracket fungi.

Mushroom is considered as proteinaceous and is applicable as food. Button mushroom or the white Mushroom are sold as fresh mushroom and they can be stored as such and are utilized as soups, sauces and other food products. Protein contents in these mushrooms are 60-70 % which is digestible and it contains all those essential amino acids and derivatives. It has plenty of medicinal properties as well. A higher amount of retene is

found in button mushroom which shows an antagonistic effect on several forms of cancer and tumors.

Spawn is the seed required for the growth of mushroom. They are vegetative mycelium isolated from a specified mushroom to be cultured on a considerable medium i.e. grain like wheat, black gram, maize, pea etc. In other words, spawn are grains expansion at mushroom mycelium. Essentially it involves preparation of pure culture of mushroom from the desired tissue/spores for the evaluation of selected cultures for high yield, better quality, and specified properties based traits. Maintenance of those cultures on artificially prepared agar medium is followed by culturing on sterilized wheat grains for its multiplication on grains. From 1652 to 1894 A.D. spawn were collected from the wild variety and were referred to as Natural spawn (from pastures & meadows) and Flake spawn (breaking of beds through which mushroom mycelium has run), Mill-track spawn (bricks dried prepared from mixture dung of cow and horse along with loamy soil). In the 20th century pure forms of mycelial culture were made and were used for preparing manure for spawn on sterilized horse manure and compost manure.

Process for making grain as spawn was first done by the Pennsylvania State University in 1932. Advantages of Grain spawn over manure spawn were it could be stirred easily and can provide many inoculation points. Grain spawn was calibrated by Stoller in 1962. Nowadays most common spawn laboratories worldwide are taking wheat as the substrate for spawn production and are following the standards of mother spawn from a pure culture of mycelium on synthetic medium.

MATERIAL AND METHODS

This work was done at *"Center for Excellence in Biotechnology Research and Training (CEBRT), Department of Biotechnology, AKS University, Satna(M.P.)"*. Primary culture of *Pleurotus* species was collected from *"Research Institute, Biodiversity Conservation and Rural Biotechnology Center (BCRBC), Jabalpur (M.P.)"*.

MULTIPLICATION OF TEST SAMPLE

A test sample (*Pleurotus* mycelium) stored at 4^0 C was multiplied by subculturing of it at Potato Dextrose Agar medium in test tubes slants. The incubation period for full growth of mycelium before transferring in spawn bottle is 25^0 C for 10 days.

OPTIMIZATION OF NUTRITIONAL REQUIREMENTS

Selection of Grains

An experiment was performed to optimize the type, quality and quantity of grains required for filling up bottles for the highest yield of spawn with adequate quality to result as to produce the edible mushrooms in Bags. Here, Variable grains were Black Gram, Peas, Maize, Barley and Wheat in 50% of the complete bottle to 80% which were HYV seeds.

Boiling and Sterilization of Grain

Grains were selected which were of HYV for 2 times washing was performed followed by boiling at 80^o C for 20 minutes. Grains are easily breakable by fingers indicate they ready to be filled in bottles. Additional moisture was separated by air drying for 2-3 hours. These dried Mushrooms were collected in a tray to add chemical for the evenness and separations all along the spawn production. Chemicals are $CaCO_3$ 10% and $CaSO_4$ 20% added to the tray with grains and mixed vigorously. Now, the bottles are autoclaved after filling grains for 45 minutes at 121^0 C temperature followed by cooling it for overnight and the next day they are ready to be exposed in front of UV radiations. Bottles are ready for inoculation of test mycelium.

Transferring of Slant into Bottles of Spawn

After UV treatment fully grown mycelium (Primary Culture of *P. florida*) were transferred into Spawn Bottles. After transferring shaking of the bottles was done for complete mixing of mycelium and the bottles kept for incubation at 20° C temperature for 21 days.

TEMPERATURE OPTIMIZATION

Wheat grains comprised in the spawn bottles were exposed to variable temperature conditions simultaneously for complete growth to form a mother culture, sister culture or planting culture such as 16° C., 18° C., 20° C., 22° C., 23° C., 25° C., 27° C. for 21days incubation

RADIATION TREATMENT

Wheat grains comprised in the spawn bottles were exposed to radiation for variable time limits between 20 to 70 minutes intervals. It was initiated to reduce the contamination during the experiment and to impart vitamin D to the grains. Change in the time optimized showed a decrease in the growth of mushrooms as in inhibition occurred.

RESULT& DISCUSSIONS

In the present investigation entitled "Optimization of growth conditions for Spawn production of *Pleurotus Florida*" various parameters i.e. nutritional requirement of secondary food, temperature, and radiation treatment have been optimized by using different culture conditions. For the optimization of nutrition requirement different types of grains has been tested as secondary food material for spawn production, in which lowest growth has been observed in Pea grains (40%), whereas wheat grains show maximum growth (95%) as shown in table-1. Wheat grains are composed of very low Saturate Fat, Cholesterol and Sodium. They are good source for Dietary Fiber, Manganese and Selenium hence these grains are suitable for high growth of spawn.

Table 1: Optimization of Nutritional Requirement

S.No.	Grains	Growth Rate
1	Black Gram	67%
2	Wheat	95%
3	Barley	45%
4	Maize	76%
5	Pea	40%

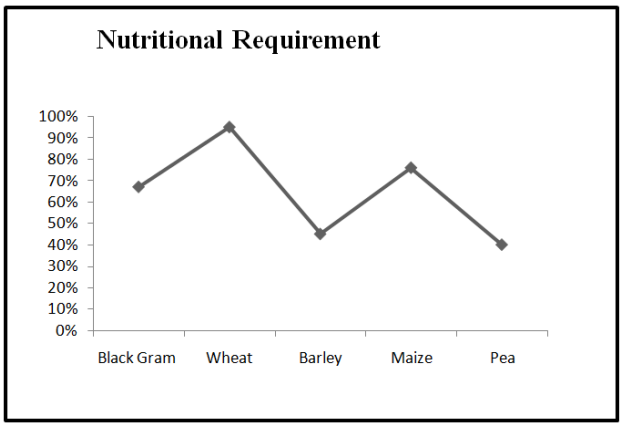

Figure 1

For the optimization of temperature, spawn bottles were incubated in different temperatures ranges from 16°C - 27°C. The minimum growth has been observed in16°C (35%) however maturation rate and progression of spawn were significantly higher at 22°C (98%) as represented in Table-2. Change of temperature during the incubation period was a critical parameter for high yield of spawn.

Table 2: Temperature Optimization

S. No.	Temperature	Growth Rate
1	16°C	35%
2	18°C	45%
3	20°C	76%
4	22°C	98%
5	23°C	75%
6	25°C	71%
7	27°C	55%

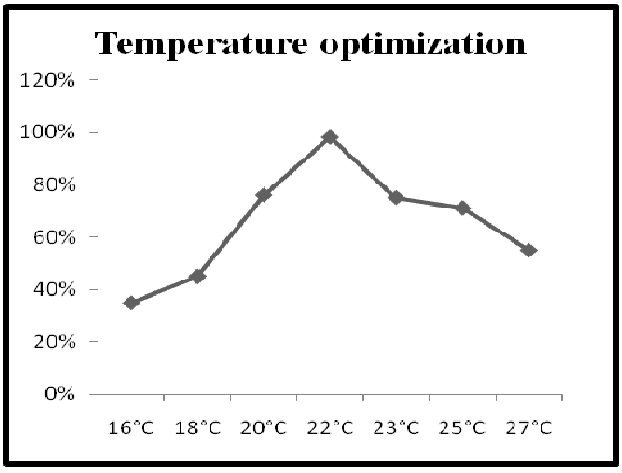

Figure 2

Mushroom is a fungus-based food product and our priority is to define it safe for human consumption. Hence, radiation-based sterilization for variable time slots had been in used to overcome the chance of contamination in spawn bottles. During the experiment, UV radiation was exposed on spawn bottle for a variable period of time ranging from 15 to 65 minutes. 15-minute exposure show the 30% efficacy and 45 minute UV treatment on grains gave the best result of 90% survival rate as shown in Table-3. UV is not only utilized for the sterilization but it also provides Vitamin D to mushrooms as reported by US researchers.

Table 3: Radiation Treatment

S. No.	Treatment Time	Efficacy
1	15 minutes	30%
2	25 minutes	55%
3	35 minutes	80%
4	45 minutes	90%
5	55 minutes	62%
6	65 minutes	45%

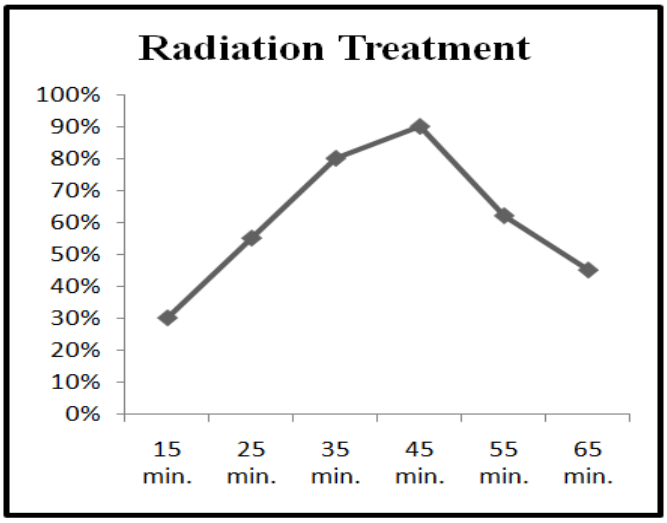

Figure 3

Optimization of oyster mushroom (*Pleurotus Florida*) on parameters like UV treatment, temperature, and the nutritional requirement was attempted primarily to determine the possibility of its better growth of spawn and cultivation of mushroom under controlled conditions. V. Karuppuraj *et.al* (2014) reported that mushroom has grown at sorghum as a substrate gave efficiency 76% and paddy straw showed maximum growth of mushrooms in controlled conditions whereas in contrast to Md. Nazim *et.al* (2007) proved that on his aspect of investigation on variable substrate showed variable result but not an optimized result was reported in his paper. We investigated that on optimization of temperature as 22°C, UV treatment for 40-50 min and substrate i.e. grain used as wheat of HYV quality obtained the better results for spawn production.

CONCLUSIONS

Pleurotus Florida is an important variety of mushroom which has a lot of medicinal and nutritive properties. At present this mushroom becomes a better alternative for overcoming the problem of malnutrition. Hence the cultivation of this mushroom is very beneficial for our society. The present study was targeted for optimization of the spawn production process which is an important step of high yield mushroom production. Therefore the result obtained during the above investigation will be very fruitful for researchers working in the area of Mushroom cultivation.

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